NREL-Amoco CRADA Phase 3

Bench Scale Report 1.6

Two-Stage Continuous Cofermentation of Pure Sugars by LNHST2

Project Title: Amoco-NREL CRADA with corn fiber

Researcher: Susan Toon @ (303) 384-6141

Director of Research: George Philippidis @384-6888

Start Date of experiment: October 24, 1995

Date of Draft Report: January 2, 1996

Date of Final Report: January 16, 1996

NREL Technical Record Book: No. 1472

Objective

(1) Examine the ability of LNHST2 to coferment glucose and xylose in a two-stage system and compare the results to those obtained with the strain L1400(pLNH33) in previous experiments; and (2) Determine the product distribution and ethanol yield for each stage and the overall process. (Note: In the rest of the report, LNHST2 is referred to as **ST2** and L1400(pLNH33) as **LNH33**.)

Background

A new yeast strain, designated ST2, with the xylose-catabolism genes incorporated into the chromosome was obtained from Nancy Ho at Purdue University. Previously, a two-stage continuous cofermentation of pure sugars was studied to determine growth characteristics, glucose and xylose utilization, and ethanol and byproduct yields for LNH33, which carries the xylose-catabolism genes on a plasmid. In order to determine if the new strain, ST2, performs better than LNH33 under identical conditions, the fermentation experiment described in Report 1.5 was repeated here with ST2.

Materials and Methods

Inoculum Preparation

The inoculum was prepared by streaking a YEPX plate (1% w/v yeast extract, 2% w/v peptone, 2% w/v xylose and 2% w/v agar, pH 5.0) from a liquid culture of ST2 received from Nancy Ho. The plate was incubated at 30°C for 96 hours. At that time, a loopfull was transferred into 25 mL of YEPX and incubated at 30°C with an agitation of 150 rpm. After 15 hours of growth, 10% v/v was transferred into 2% w/v Corn Steep Liquor (CSL), 1% w/v yeast extract, and 2% w/v xylose at pH 5.0 for inoculum growth. This 500-mL baffled Erlenmeyer flask contained a working volume of 100 mL and again was incubated at 30°C with an agitation of 150 rpm. After 21 hours of incubation, the culture was used to inoculate the first stage fermentor.

Fermentation Conditions and Configuration

The fermentation conditions and configuration were identical to those reported in Report 1.5, entitled *Two-Stage Continuous Cofermentation of Pure Sugars by L1400(pLNH33):*

For the fermentations, two 1.7-L New Brunswick BioFlo III fermentors were employed. To minimize ethanol evaporation, the condensers on each unit were packed with 1-mm glass beads (to maximize the surface area) and equipped with 4°C-water circulation. The working volume of each vessel was one liter, agitation was controlled at 150 rpm, temperature was maintained at 30°C, and the pH was maintained at 5.0 with the addition of 3 M sodium hydroxide. Air was not supplied to the fermentors.

The first stage fermentor was started in batch mode with 2% w/v CSL, 1% w/v yeast extract, 2.4% w/v glucose, and 3.4% w/v xylose as the medium. The first stage fermentor was prepared and autoclaved with CSL, water, and yeast extract at pH 5.0. Stock solutions of glucose and xylose were filter sterilized separately from the fermentor and added to the fermentor with the inoculum (to avoid Maillard reactions). A 10% v/v inoculum was transferred to the fermentor vessel and was allowed to grow for 24 hours in batch mode before being switched to continuous operation. The effluent from the first stage was directed to the second stage (Figure 1). The feed for the continuous mode consisted of the same medium as the reactor, but was made up in a 15-L vessel with xylose and glucose being filter sterilized and added after the yeast extract and CSL solution was autoclaved.

The second stage was sterilized with enough water to cover the pH probe membrane. After sterilization and before the effluent line from the first stage was connected, a majority of the water was pumped out of the fermentor. The residence for the first stage was set at 24 hours; hence, once the second stage was attached to the system, it took 24 hours to fill it to the one-liter working volume.

The feed, base, and acid addition vessels were placed on balances and the weights were recorded daily in order to calculate the dilution rate for each fermentation and the overall dilution rate. The dilution rate was calculated by dividing the weight change over time of the feed (the base addition was negligible) by the working volume of the fermentor (density of feed assumed to be 1.0 g/mL). The residence time is the inverse of the dilution rate.

Sampling and Analysis

Samples were withdrawn at regular intervals and analyzed on the Yellow Springs Instrument (YSI) for ethanol and glucose. In addition, samples were analyzed by the Chemical Analysis and Testing (CAT) Team for glucose, xylose, apparent xylitol, acetic acid, lactic acid, and glycerol by HPLC and ethanol by GC. Optical density at 600 nm (OD) and dry cell weight were obtained on every sample to monitor cell growth. The dry cell weight was determined by centrifuging 4 mL of the fermentation broth in duplicate for 10 minutes at 5000 rev/min. The supernatant was decanted and the pellet was washed with 10 mL of deionized water twice. The pellets were then transferred to weighed pans and let to dry in a 60°C drying oven for 24 hours.

Results and Discussion

Stage One

In the previous two continuous fermentation experiments with LNH33 (Reports 1.2 and 1.5), a span of residence times from 24 hours to 74 hours was examined. The target residence time for each stage in this experiment was 24 hours. The actual (calculated) residence time was 23 hours per stage resulting in an overall residence time of 46 hours. Testing the two organisms under similar residence times will allow direct comparison of the results.

The majority of the glucose (over 94%) was consumed within eight hours and all of it was depleted within 24 hours in batch mode (Table 1, batch 3 column). The residual glucose remaining at eight hours may be due to the fact that the inoculum was slightly older than in previous experiments, indicating the lag that can occur when older cultures are used as inoculum. Within 24 hours, xylose dropped to 8.39 g/L from an

original concentration of 32.8 g/L. This rate seems to be slightly faster than the rate observed with LNH33 (Table 1). After 24 hours of growth in batch mode, the fermentation was switched to continuous with a feed rate of 0.716 mL/min, yielding a 23-hour residence time.

Table 1: Comparison of the sugar concentration profiles during the batch phase of the previous two experiments with LNH33 (batch 1 and 2) and this experiment with ST2 (batch 3)[1].

	Glucose (g/L)			Xylose (g/L)		
Time (h)	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
0	25.51	25.5	24.75	38.44	35.40	32.80
6	4.43	3.68	10.88	37.77	35.47	33.26
8		0.00	1.40		32.36	31.16
24	0.00	0.00	0.00	15.8	13.62	8.39

[1] Batch 1 and 2 data are from Reports 1.2 and 1.5, respectively.

After the fermentation was switched to continuous operation, the glucose concentration remained at zero. The xylose concentration increased from 8.39 g/L to 13.4 g/L, yielding a utilization level of 58.3% of the feed xylose. This represents a significant improvement of xylose utilization over LNH33, which only used 11.3% of the xylose (feed levels similar in both experiments) in the first stage at a 24-hour residence time.

The ethanol and byproduct yields and the glucose and xylose conversions for stage one were calculated based on the average data from time points 146 through 265 (hours). The metabolic ethanol yield (based on consumed sugars) for stage 1 was 77.9% of theoretical, whereas the ethanol process yield (based on the available fermentable sugars) was lower at 58.7% of theoretical (Table 2) due to incomplete xylose consumption.

Table 2: Fermentation performance at the 23-hour residence time per stage

Stage	1	2	Overall
Residence Time	23	23	46
C6-Conversion:	99.5%	100.0%	100.0%
C5-Conversion:	58.3%	67.4%	86.4%
Ethanol Process Yield (% theoretical):	58.7%	47.1%	70.3%
Ethanol Metabolic Yield (% theoretical):	77.9%	69.7%	76.4%

In the first stage, glycerol, cell mass, and apparent xylitol were the major byproducts (Table 3). The product distribution for ST2 in stage one is quite different from that observed for LNH33, where only minor amounts of glycerol were produced (see Report 1.5). This may be due to the higher xylose utilization in stage one by ST2, which results in a concomitant increase in glycerol production (related pathways). The same phenomenon was observed in the second stage of the LNH33 continuous

fermentation (Report 1.5), where xylose was the only available sugar source. The overall carbon balance closure for the first stage was excellent at 101.3%.

Table 3: Product Distribution

	g product/100 g C6+C5 consumption		
	Stage 1	Stage 2	Overall
Ethanol	39.85	35.63	39.09
Cell Mass	7.12	6.67	7.04
Carbon Dioxide	38.07	34.04	37.34
Glycerol	10.89	2.51	9.37
Lactic Acid	0.25	0.00	0.00
Apparent Xylitol	5.10	15.63	7.02
Total	101.29	94.47	99.85

Stage Two and Overall

Stage two was completely filled and operational after 50 hours from the time stage one was inoculated. The only fermentable sugar available in stage two was xylose, since all glucose was consumed in the first stage. Unlike the fermentation with LNH33, where the residual xylose concentration increased drastically in the second stage due to the organism's poor ability to ferment xylose (resulting in an overall xylose conversion of only 27.1%), the xylose level here decreased to a low level of 4.37 g/L and remained steady (Figure 2 and 4). This result demonstrates the ability of ST2 to retain its ability to ferment xylose over an extended time frame. In contrast, LNH33 did not reach steady state even after 288 hours of continuous operation (Report 1.5). In stage two, 67.4% of the xylose was consumed yielding an overall process conversion of 86.4% within a 46-hour residence time.

The ethanol process yield was 47.1% of theoretical in the second stage and 70.3% of theoretical overall (Table 2). The ethanol metabolic yield was 69.7% in the second stage and 76.4% overall (Table 2). These yields are slightly lower than those observed with LNH33. This may be due to the larger production of glycerol by ST2 (4.89 g/L) compared to LNH33 (1.41 g/L) as a result of the higher xylose consumption by ST2. Besides glycerol, the other major byproducts were apparent xylitol and cell mass (Table 3).

Switching to a 24-hour overall residence time

After a steady state was achieved at an overall residence time of 46 hours, the residence time was decreased (at 360 hours) from 24 to 12 hours per stage for an overall residence time of 24 hours. At this fast pace, all of the glucose (24.9 g/L) was still utilized in the first stage, but the xylose concentration increased to 26.5 g/L in stage one and 16.6 g/L in stage two, before the experiment was terminated (unfortunately before a new steady state was reached).

Interestingly, the xylose consumption in stage one at the 23-hour residence time was 18.74 g/L, close to the 19.04 g/L observed at an overall residence time of 24 hours. Stage one produced 16.4 g/L of ethanol in 23 hours compared to 17.5 g/L produced through both stages at the 24-hour residence time. The similarity in data between the one stage at a 23 hour residence time and the two-stage system at a 24-hour residence

time again shows that there may not be an advantage to a two-stage system with the pure sugar fermentation.

Conclusions

The most encouraging result from this experiment is the 86.4% conversion of xylose by ST2 at a residence time of 46 hours coupled with the constant utilization of xylose over an extended time period (Figure 4). The ethanol metabolic yield was good at 76.4% of theoretical (22 g/L). On the negative side, the production of glycerol and xylitol was greater with ST2 than with LNH33 and is probably due to the increased amount of xylose utilized by ST2. It is an important issue that should be kept in mind during scale-up and process design, because glycerol and xylitol represent significant "waste products" for the biomass-to-ethanol process (16.4% of the sugar carbon in this study) and should be minimized.

Figure 1: Pure Sugar Continuous Cofermentation by LNHST2
First Stage

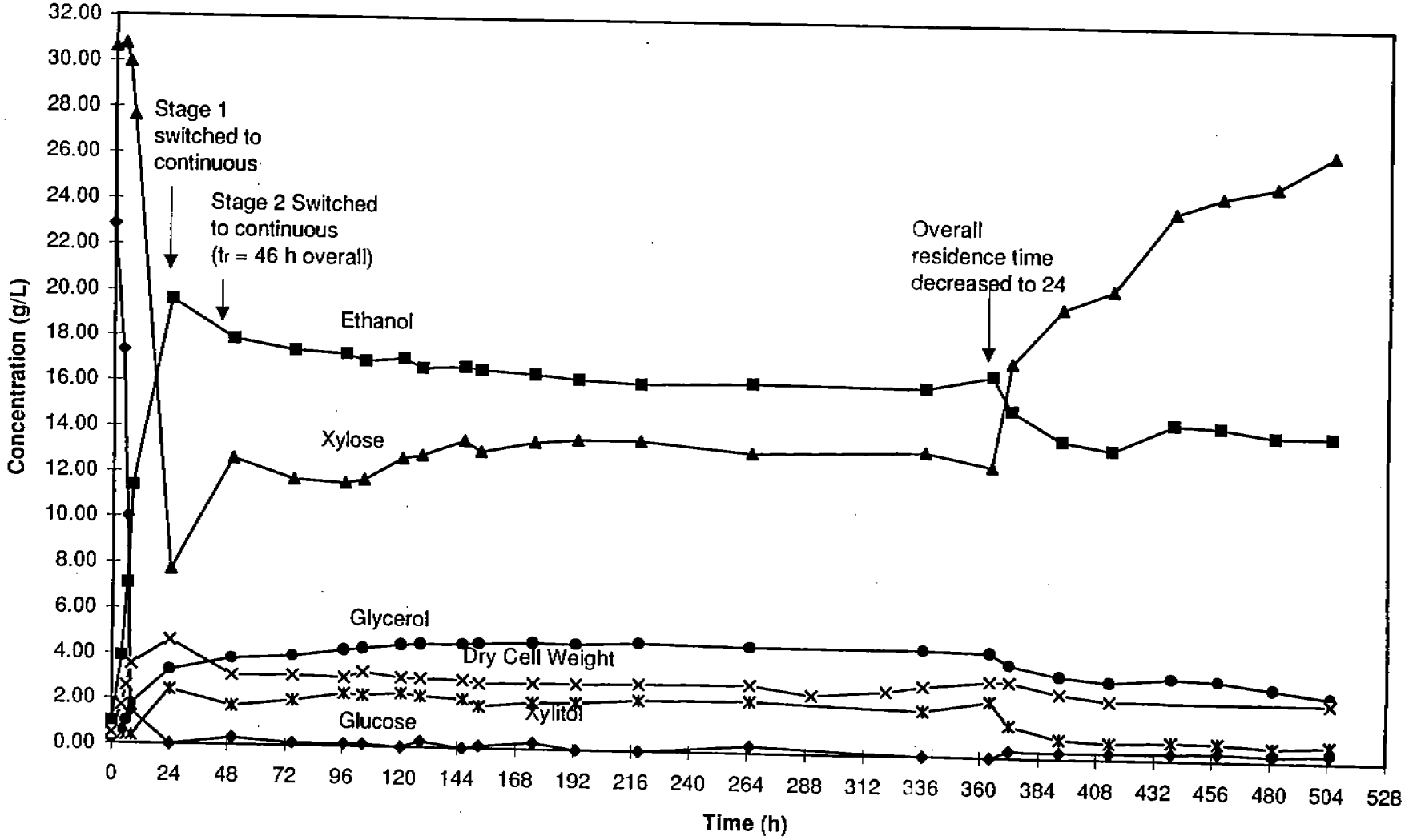


Figure 2: Pure Sugar Continuous Cofermentation by LNHST2
Second Stage

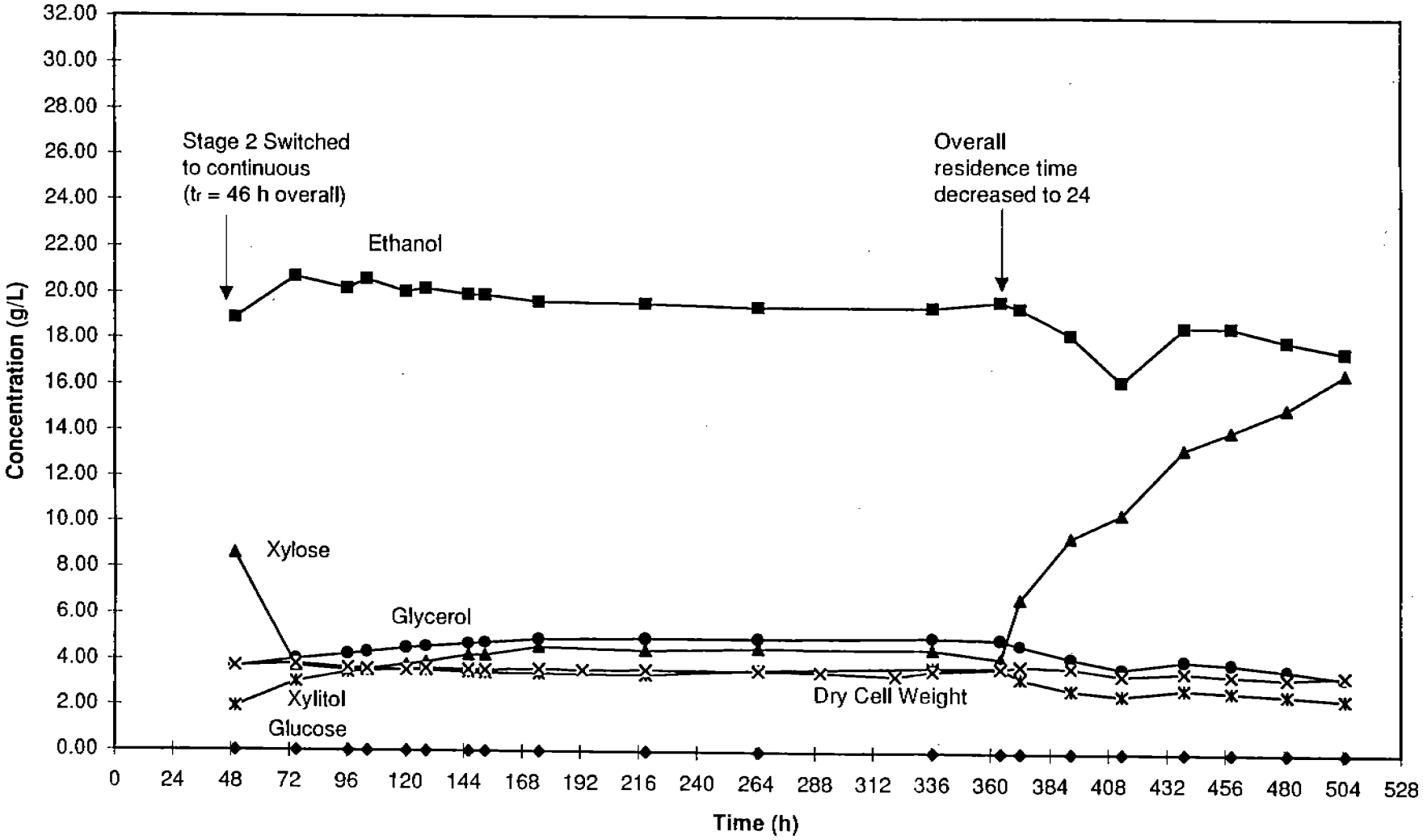


Figure 3: Pure Sugar Continuous Cofermentation by LNHST2
Two Stages

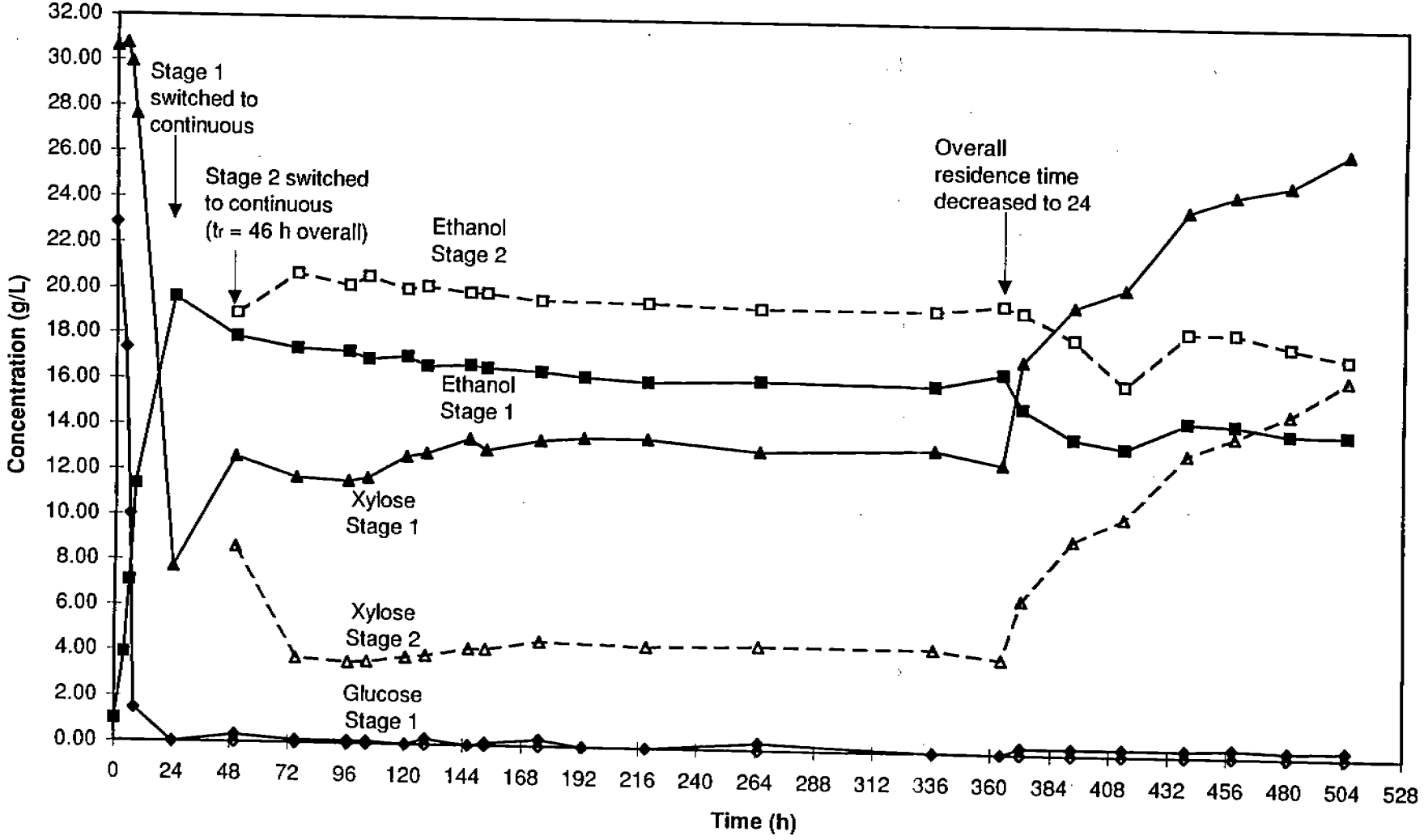
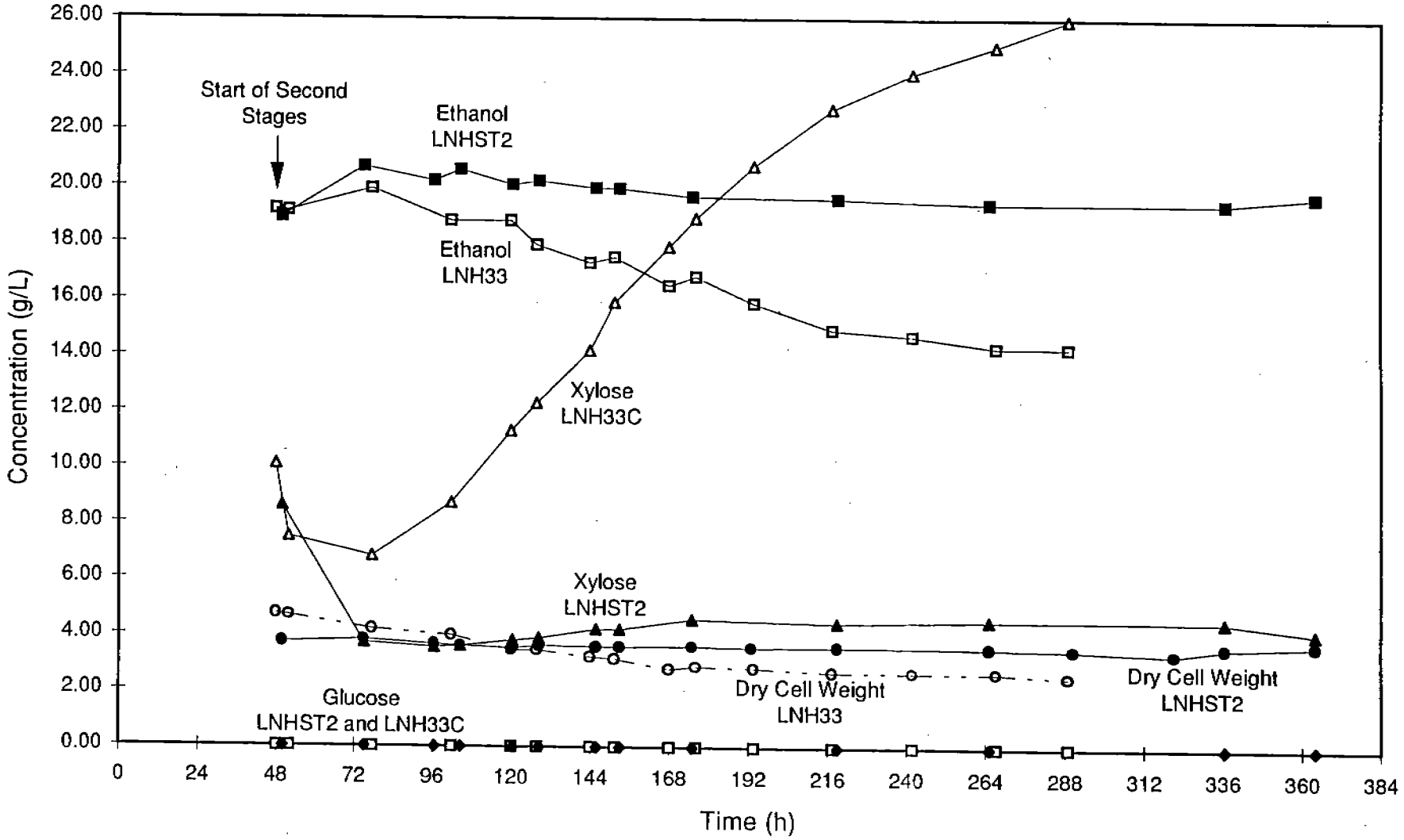


Figure 4: Comparison of L1400(pLNH33) and LNHST2 Second Stage Data



Raw Data

		Stage 1									
				YSI	LC	YSI	LC				
Sample	Time elapsed (h)	OD (600 nm)	DCW (g/L)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Xylitol (g/L)	Glycerol (g/L)	Lactic acid (g/L)
1	0	0.90	0.50	24.50	22.86	0.76	0.98	30.61	0.37	0.25	2.90
2	4	2.25	1.70	19.60	17.33	3.56	3.88	30.73	0.37	0.56	2.87
3	6	3.73	2.56	10.94	10.01	7.56	7.08	29.94	0.37	1.00	2.87
4	8	5.36	3.51	1.54	1.46	12.56	11.36	27.61	0.39	1.76	2.86
5	24	8.38	4.59	0.03	0.00	20.81	19.60	7.71	2.41	3.28	2.81
6	50	4.86	3.08	0.15	0.32	19.78	17.88	12.59	1.71	3.82	2.73
7	75	4.48	3.09	0.15	0.12	18.92	17.38	11.69	1.99	3.95	2.55
8	96.5	4.56	3.04	0.10	0.11	18.80	17.25	11.56	2.32	4.23	2.56
9	104.5	4.34	3.28	0.16	0.12	19.30	16.94	11.71	2.25	4.32	2.57
10	120.5	4.20	3.05	0.10	0.00	19.10	17.07	12.69	2.36	4.50	2.62
11	128.5	4.27	3.04	0.08	0.26	18.52	16.66	12.84	2.26	4.55	2.62
12	146	3.94	2.99	0.13	0.00	18.04	16.73	13.50	2.13	4.57	2.62
13	153	3.95	2.86	0.10	0.11	18.44	16.62	13.03	1.84	4.61	2.55
14	175	3.92	2.89	0.09	0.29	16.91	16.48	13.48	2.06	4.68	2.61
15	193	4.24	2.90	0.14	0.00	17.56	16.27	13.62	2.08	4.67	2.62
16	219	4.15	2.95	0.10	0.00	16.90	16.11	13.62	2.22	4.75	2.66
17	265	4.21	2.98	0.15	0.31	17.80	16.20	13.14	2.28	4.66	2.58
18	290.5	3.52	2.58	0.23		18.10					
19	321.25	3.64	2.80	0.21		18.60					
20	336.5	4.08	3.06	0.07	0.00	17.00	16.11	13.30	1.98	4.65	2.55
21	364	4.53	3.33	0.09	0.00	18.02	16.66	12.70	2.45	4.59	2.66
22	372	4.61	3.31	0.14	0.30	16.01	15.18	17.24	1.44	4.07	2.66
23	392.5	3.97	2.84	0.31	0.30	15.18	13.88	19.68	0.87	3.60	2.50
24	413.5	3.68	2.54	0.32	0.29	13.68	13.51	20.50	0.74	3.38	2.45
25	439	4.22		0.49	0.30	15.04	14.65	23.93	0.80	3.60	2.78
26	458.5	3.52	2.59	0.46	0.37	15.29	14.55	24.62	0.78	3.50	2.78
27	481	3.66	2.60	0.47	0.26	14.54	14.17	25.09	0.63	3.17	2.67
28	505	3.65	2.53	0.43	0.33	14.00	14.16	26.50	0.71	2.81	2.78
29	528.5	3.53		0.45		13.63					

Raw Data

		Stage 2									
				YSI	LC	YSI	LC				
Sample	Time elapsed (h)	OD (600 nm)	DCW (g/L)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Xylitol (g/L)	Glycerol (g/L)	Lactic acid (g/L)
1	0										
2	4										
3	6										
4	8										
5	24										
6	50	5.62	3.73	0.04	0.00	20.88	18.90	8.60	1.93	3.68	2.68
7	75	5.49	3.81	0.03	0.00	21.80	20.70	3.71	3.02	4.02	2.58
8	96.5	5.12	3.65	0.04	0.00	21.80	20.19	3.55	3.45	4.24	2.55
9	104.5	4.70	3.60	0.03	0.00	22.00	20.60	3.60	3.53	4.32	2.54
10	120.5	4.71	3.54	0.04	0.00	22.48	20.06	3.80	3.56	4.49	2.54
11	128.5	4.68	3.63	0.03	0.00	21.40	20.19	3.89	3.53	4.57	2.52
12	146	4.67	3.59	0.02	0.00	20.80	19.95	4.21	3.45	4.70	2.52
13	153	4.66	3.58	0.03	0.00	21.40	19.93	4.22	3.43	4.75	2.51
14	175	4.52	3.60	0.03	0.00	22.00	19.64	4.57	3.42	4.91	2.54
15	193		3.56	0.14		20.80					
16	219	4.75	3.59	0.02	0.00	20.60	19.57	4.44	3.37	4.94	2.51
17	265	5.28	3.55	0.03	0.00	21.00	19.41	4.53	3.58	4.95	2.53
18	290.5	4.70	3.50	0.06		21.50					
19	321.25	4.91	3.35	0.06		21.00					
20	336.5	4.78	3.58	0.02	0.00	19.16	19.41	4.51	3.72	5.02	2.56
21	364	5.27	3.70	0.01	0.00	20.60	19.67	4.12	3.75	4.95	2.57
22	372	5.41	3.80	0.02	0.00	19.09	19.38	6.71	3.26	4.70	2.57
23	392.5	4.97	3.75	0.01	0.00	18.02	18.24	9.38	2.77	4.17	2.53
24	413.5	5.09	3.40	0.03	0.00	17.22	16.23	10.41	2.55	3.71	2.41
25	439	5.47	3.55	0.11	0.00	18.08	18.57	13.25	2.80	4.05	2.72
26	458.5	4.96	3.40	0.07	0.00	17.44	18.57	14.05	2.70	3.91	2.72
27	481	5.03	3.30	0.39	0.00	17.18	17.97	15.04	2.56	3.66	2.67
28	505	4.91	3.40	0.03	0.00	16.89	17.47	16.55	2.39	3.33	2.68
29	528.5	4.95		0.03		16.54					

CARBON BALANCE: LNHST2 Continuous Cofermentation with Pure Sugars

Sample: First stage - 23 hour residence time
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	99.5%
Overall C5-Sugar Conversion:	58.3%
Ethanol Process Yield (% theor):	58.7%
Ethanol Metabolic Yield (% theor):	77.9%

Carbon Balance: SSF

	Carbon In							Carbon Out						Conversion	Yield	Yield
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	(In-Out)/In	g product/	g product/
Component	(% dry wt)	(C-mole/Kg Sl	(% Total In)	(g/L)	(C-mole/Kg Sl	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sl	% Total Out)	(g/L)	(C-mole/Kg Sl	% Total Out)	(C-mole/Kg Sl	(%)	100 g C6 cons	100 g C6+C5 cons
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0	0.000	*0.0*	22.53	0.750	*100.0*	0.750	0.00	0.000	*0.0*	0.12	0.004	*100.0*	0.004	99.47		
Galactose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Mannose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Xylose	0	0.000	*0.0*	32.14	1.070	*100.0*	1.070	0.00	0.000	*0.0*	13.40	0.446	*100.0*	0.446	58.31		
Arabinose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Lignin	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
	0																
Ethanol				0.00	0.000		0.000				16.40	0.712		0.712		73.18	39.85
Cell Mass				0.00	0.000		0.000				2.93	0.117		0.117		13.07	7.12
Carbon Dioxide				0.00								0.356		0.356		69.91	38.07
Glycerol				0.18	0.006		0.006				4.66	0.152		0.152		20.00	10.89
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Lactic Acid				2.51	0.083		0.083				2.61	0.087		0.087		0.46	0.25
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Xylitol				0.00	0.000		0.000				2.10	0.069		0.069		9.37	5.10
Other																	
Total	*0*	0.000	*0.0*		1.910	*100.0*	*1.910*	*0.00*	*0.000*	*0.0*		1.943	*100.0*	*1.943*		*176.63*	*101.29*

C-RECOVERY: 101.72%

Component	% Carbon In	% Carbon Out
Glucose	39.3%	0.2%
Galactose+Mannos	0.0%	0.0%
Total C5 Sugars	56.0%	23.0%
Lignin	0.0%	0.0%

CARB[redacted]ALANCE: LNHST2 Continuous Cofermentation with Pure Sugars

Sample: Stage 2 - 23 hr residence time, overall = 46 hr
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	67.4%
Ethanol Process Yield (% theor):	47.1%
Ethanol Metabolic Yield (% theor):	69.7%

Carbon Balance: SSF

	Carbon In						Carbon Out								
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	Conversion	Yield	Yield
Component	(% dry wt)	(C-mole/Kg Sl	(% Total In)	(g/L)	(C-mole/Kg Sl	(% Total In)	(C-mole/Kg Sl)	(% dry wt)	(C-mole/Kg Sl | % Total Out)	(g/L)	(C-mole/Kg Sl | % Total Out)	(C-mole/Kg Sl	(In-Out)/In (%)	g product/ 100 g C6 cons	g product/ 100 g C6+C
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0	0.000	0.0	0.12	0.004	100.0	0.004	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.00		
Galactose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0	0.000	0.0	13.40	0.446	100.0	0.446	0.00	0.000	0.0	4.37	0.146	100.0	0.146	67.39		
Arabinose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol	0																
Cell Mass				16.40	0.712		0.712				19.66	0.853		0.853		2716.67	35.63
Carbon Dioxide				2.93	0.117		0.117				3.54	0.141		0.141		508.33	6.67
Glycerol				0.00								0.071		0.071		2595.21	34.04
Acetic Acid				4.66	0.152		0.152				4.89	0.159		0.159		191.67	2.51
Lactic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Succinic Acid				2.61	0.087		0.087				2.53	0.084		0.084		-66.67	0.00
Xylitol				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Other				2.10	0.069		0.069				3.53	0.116		0.116		1191.67	15.63
Total	0	0.000	0.0		1.587	100.0	1.587	0.00	0.000	0.0		1.571	100.0	1.571		5945.21	94.47

C - Recovery: 98.97%

Component	% Carbon In	Out
Glucose	0.3%	0.0%
Galactose+Mannose	0.0%	0.0%
Total C5 Sugars	28.1%	9.3%

CARBON BALANCE: LNHST2 Continuous Cofermentation with Pure Sugars

Sample: Overall - 46 hr residence time
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	86.4%
Ethanol Process Yield (% theor):	70.3%
Ethanol Metabolic Yield (% theor):	76.4%

Carbon Balance: SSF

	Carbon In						Carbon Out						Conversion	Yield	Yield
	In Solids			In Liquor		Total	In Solids			In Liquor		Total	(In-Out)/In	g product/	g product/
Component	(% dry wt)	(C-mole/Kg Sln	(% Total In)	(g/L)	(C-mole/Kg Sln	(% Total In)	(C-mole/Kg Sln	(% Total Out)		(g/L)	(C-mole/Kg Sln	(% Total Out)	(C-mole/Kg Sln	(%)	100 g C6 cons	100 g C6+C5 cons
Cellobiose				0.00	0.000		0.000									
Glucose	0	0.000	*0.0*	22.53	0.750	*100.0*	0.750	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	100.00		
Galactose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Mannose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Xylose	0	0.000	*0.0*	32.14	1.070	*100.0*	1.070	0.00	0.000	*0.0*	4.37	0.146	*100.0*	0.146	86.40		
Arabinose	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
Lignin	0	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	0.00	0.000	*#DIV/0!*	0.00	0.000	*#DIV/0!*	0.000	#DIV/0!		
	0																
Ethanol				0.00	0.000		0.000				19.66	0.853		0.853		87.26	39.09
Cell Mass				0.00	0.000		0.000				3.54	0.141		0.141		15.71	7.04
Carbon Dioxide				0.00								0.427		0.427		83.36	37.34
Glycerol				0.18	0.006		0.006				4.89	0.159		0.159		20.91	9.37
Acetic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Lactic Acid				2.51	0.083		0.083				2.53	0.084		0.084		0.10	0.00
Succinic Acid				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Xylitol				0.00	0.000		0.000				3.53	0.116		0.116		15.67	7.02
Other																	
Total	*0*	0.000	*0.0*		1.910	*100.0*	*1.910*	*0.00*	*0.000*	*0.0*		1.927	*100.0*	*1.927*		*207.35*	*99.85*

C-RECOVERY: *100.87%*

Component	% Carbon In	% Carbon Out
Glucose	39.3%	0.0%
Galactose+Mannose	0.0%	0.0%
Total C5 Sugars	56.0%	7.6%
Lignin	0.0%	0.0%
Ethanol	0.0%	44.3%
Byproducts	4.7%	42.1%